Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the investigation of **Potassium Metabisulfite** in the developing chicken embryo 1/31/74

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of POTASSIUM METABISULFITE in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken Embryo:

POTASSIUM METABISULFITE

PROTOCOL:

Potassium metabisulfite (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered, with water as the diluent, by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3).

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment. In addition, a small number of pierced and drilled controls were collected in some experimental groups.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated. This number has not been corrected for the sterile eggs or the eggs discarded due to accidents, thus providing a slightly higher mortality rate and a lower abnormality rate than was the actual case.

Column 4 is the percent mortality, i.e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

The comparable data for the solvent treated eggs and the pierced and drilled controls as well as the untreated controls are included in columns 3 through 6.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Potassium metabisulfite was found to be quite embryotoxic when administered to the embryos under all of the conditions of the test; the yolk treatment was more toxic than the air cell treated eggs. The toxicity in the latter was significantly ($P=0.05$) greater than it was in solvent-treated eggs at and above the dosage of 1.0 mg/egg, except at the dosages of 2.5 mg/egg and .25 mg/egg at 96 hours (Tables 1 and 2). In the yolk treated eggs, however, the toxicity was significantly greater than it was in the control eggs at all the tested dose levels (Tables 3 and 4). Probit analysis resulted in an LC_{50} of 0.526 mg/egg (air cell at 0 hours) and an LC_{50} of 0.923 mg/egg (air cell at 96 hours). Analysis of the yolk treatment showed a negative slope for both the 0 hours and the 96 hours eggs.

Abnormal birds were seen under all conditions of the test, especially in the air cell treated birds. The incidence of birds having a structural abnormality of head, limbs, viscera, or skeleton was not significantly different from the solvent background ($P=0.05$). Of the 91 untreated control embryos, only one had the minor abnormality of curled toes.

AIR CELL AT 0 HOURS: At 5.0 mg/egg one bird was found with celosomia. At 2.5 mg/egg two birds were abnormal, one with a short beak

and the other with talipes. At 1.0 mg/egg one bird showed celosomia. At 0.5 mg/egg two birds had celosomia and a third was found to have curled toes. At 0.25 mg/egg, where a total of four abnormal birds were seen, two birds had celosomia and another pair had hip contracture. Only one of the solvent-treated birds was found to have an abnormality, celosomia.

AIR CELL AT 96 HOURS: At .25 mg/egg there were two abnormal birds showing either buphthalmia or curled toes. At 10 mg/egg one bird had hip contracture and a second bird displayed celosomia with a short beak. At 2.5 mg/egg celosomia was seen in one bird.

There were a total of seven abnormal birds at 1.0 mg/egg; abnormalities seen included celosomia, shoulder contracture, dysgnathia, short beak, and curled toes. At 0.5 and 0.25 mg/egg two and seven birds, respectively, were abnormal, all of them with curled toes. Curled toes were also found with one of the solvent-treated birds. In none of the treated cases, however, was the incidence of abnormal birds significantly different from that in the solvent-treated eggs.

YOLK AT 0 HOURS: At 10 mg/egg one bird had celosomia. At 5.0 mg/egg two birds showed hip contracture. An abnormal leg fracture was found on the one abnormal bird of the dose at 0.25 mg/egg. Only one bird, with curled toes, was found from the solvent-treated eggs to be abnormal.

YOLK AT 96 HOURS: At 10 mg/egg three birds were found to be abnormal with either hip contracture or curled toes. At 5.0 mg/egg one bird had curled toes. No abnormalities were found in the birds from the lower dose levels. Solvent-treated birds, however, revealed two birds with curled toes and one with hip contracture.

From these results it cannot be concluded that potassium metabisulfite is teratogenic to the chick embryo. The abnormalities found with the 0 hours and the 96 hours yolk treatments for the dose levels of 5 mg/egg and 10 mg/egg were almost identical to those found with the solvent-treated eggs. The air cell treatment, especially at 96 hours, exhibited more varieties of abnormalities at all the dose levels except 5.0 mg/egg at 96 hours. Statistically, however, the air cell treatment was not significant. For this reason, further experimentation, with dosage levels ranging from a high dose of 1/10 of the LC_{50} dose to a low dose of some multiple of the proposed level for human food, is recommended.

Histological examinations of the major organs revealed no evidence of consistent change due to either the dose level of the substance administered or the mode of the treatment.

1. Potassium Metabisulfite ($K_2S_2O_5$), Lot #ZPA, Mallinckrodt Chemical Works, St. Louis, Missouri, FDA 71-21

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Potassium Metabisulfite

Air Cell at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	78	100*	0	0
5.0	100	79	100*	2.53	1.26
2.5	50	80	97.50*	2.50	2.50
1.0	20	75	100*	1.33	1.33
0.5	10	78	85.89	5.12	3.84
0.25	5	78	83.33	5.12	5.12
Water		78	79.48	1.28	1.28
Control		91	41.75	1.09	1.09

LC_{30} 0.329 mg/egg (6.58 mg/kg)

LC_{50} 0.526 mg/egg (10.52 mg/kg)

LC_{90} 1.655 mg/egg (33.10 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Potassium Metabisulfite

Air Cell at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
25.0	500	37	81.08	5.40	5.40
10.0	200	80	95.0*	3.75	2.50
5.0	100	75	96.0*	0	0
2.5	50	40	65.0	10.0	5.0
1.0	20	77	89.61*	10.38	9.09
0.5	10	75	77.33	2.66	2.66
0.25	5	76	71.05	9.21	9.21
Water		81	69.13	2.46	1.23
Control		91	41.75	1.09	1.09

LC_{30} 0.374 mg/egg (7.48 mg/kg)

LC_{50} 0.923 mg/egg (18.87 mg/kg)

LC_{90} 8.391 mg/egg (167.83 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 3

Potassium Metabisulfite

Yolk at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	69	98.55*	1.44	1.44
5.0	100	77	96.10*	5.19	2.59
2.5	50	73	98.63*	0	0
1.0	20	73	100*	0	0
0.5	10	65	96.92*	0	0
0.25	5	73	97.26*	1.36	1.36
Water		52	71.15	5.76	5.76
Control		91	41.75	1.09	1.09

Slope is negative

*Significantly different from solvent $P \leq 0.05$